
AKT INHIBITORS, PHARMACEUTICAL COMPOSITIONS, AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 60/407,239, filed September 3, 2002, the disclosure of which is incorporated by reference.

FIELD OF THE INVENTION

[0002] This invention pertains to inhibitors of the serine/threonine kinase Akt, pharmaceutical compositions, and a method of preventing or treating of diseases activated by Akt.

BACKGROUND OF THE INVENTION

[0003] Akt (or protein kinase B (PKB)) is a well-characterized serine/threonine kinase that promotes cellular survival. Akt is activated in response to many different growth factors, including IGF-I, EGF, bFGF, insulin, interleukin-3, interleukin-6, heregulin, and VEGF (1). Akt is the cellular homologue of the product of the *v-akt* oncogene (2-4) and has 3 isoforms, Akt 1, 2, and 3 (or PKB α , β , and γ). Activation of all three isoforms is similar in that phosphorylation of two sites, one in the activation domain and one in the C-terminal hydrophobic motif, are necessary for full activity.

[0004] For Akt 1, phosphorylation of T308 in the activation domain by PDK1 is dependent on the products of phosphatidylinositol (PI) 3-kinase (PI3-K), phosphatidylinositol 3,4 bisphosphate (PIP₂) and phosphatidylinositol 3,4,5 trisphosphate (PIP₃). Cellular levels of PIP₂ and PIP₃ are controlled by the tumor suppressor, dual-phosphatase PTEN, which dephosphorylates PIP₂ and PIP₃ at the 3'-position. The mechanism of S473 phosphorylation is less clear. Kinases potentially responsible for S473 phosphorylation include PDK1 (5), ILK or an ILK- associated kinase (6, 7), Akt itself (8) or an as yet uncharacterized PDK2. Akt activation may also be achieved through PI3-K independent means, by phosphorylation of Akt by kinases such as PKA (9) or CAM-KK (10). Once activated, Akt exerts anti-apoptotic effects through phosphorylation of substrates that directly regulate the apoptotic machinery such as Bad (11, 12) or caspase 9 (13), or phosphorylation of substrates that indirectly inhibit apoptosis such the human telomerase reverse transcriptase subunit (hTERT) (14), forkhead transcription family members (15, 16), or I κ B kinases (17, 18).

[0005] Functionally, Akt promotes survival *in vitro* when cells are exposed to different apoptotic stimuli such as GF withdrawal, UV irradiation, matrix detachment, cell cycle discordance, DNA damage, and administration of anti-Fas antibody, TGF- β , glutamate, or

bile acids (19-33). *In vivo*, activation of the PI3K/Akt pathway contributes to tumorigenesis in many types of tissues, including breast, ovarian, brain, prostate, and lymph tissues (34). It has been shown that Akt is constitutively active in over 90% of NSCLC cell lines and contributes to both chemotherapeutic resistance and radiation resistance (35). In addition, it has been shown that Akt is constitutively active in many breast cancer cell lines, and serves a similar function in promotion of cellular survival and chemotherapeutic resistance (36).

[0006] The foregoing shows that inhibitors of Akt would be desirable for preventing or treating a number of diseases, especially diseases such as cancer.

[0007] The advantages of the invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

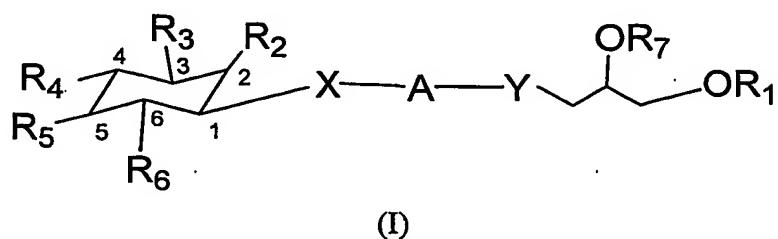
[0008] The invention provides compounds which are inhibitors of the Akt, pharmaceutical compositions comprising such an inhibitor and a pharmaceutically acceptable carrier, and a method of preventing or treating diseases by the use of such inhibitors. The inhibitors include phosphoinositol ether lipid analogues as well as bioisosteres thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0009] Figure 1A depicts the formulas of some of the Akt inhibitors.
- [0010] Figure 1B depicts the formulas of some other Akt inhibitors.
- [0011] Figure 1C depicts the formulas of yet other Akt inhibitors.
- [0012] Figure 1D depicts some of the immunoblots of the Akt inhibitors on S473 phosphorylation and total Akt levels.
- [0013] Figure 1E depicts the effect of some of the Akt inhibitors on Akt kinase activity.
- [0014] Figure 2A depicts the dose response curve for some of the Akt inhibitors.
- [0015] Figure 2B depicts the dose response curves for some of the inhibitors on Akt phosphorylation.
- [0016] Figure 3A depicts selectivity data for some of the Akt inhibitors on H1703 cells.
- [0017] Figure 3B depicts selectivity data for some of the Akt inhibitors on H157 cells.
- [0018] Figure 3C depicts selectivity data for some of the Akt inhibitors (SH 23-25) on H1703 cells.
- [0019] Figure 3D depicts selectivity data for some of the Akt inhibitors on MB468 cells.
- [0020] Figure 4 depicts the increase of apoptosis by Akt inhibitors on cells with high levels of Akt activity.

DETAILED DESCRIPTION OF THE INVENTION

[0021] The present invention provides a compound of the formula I:



or a pharmaceutically acceptable salt thereof;

wherein X and Y are independently selected from the group consisting of O, CF₂, CH₂, and CHF;

A is independently selected from the group consisting of P(O)OH, CH₂COOH, and CH(COOH)₂;

R₂ is selected from the group consisting of H, OH, isosteres of OH, C₁-C₂₅ alkyloxy, C₆-C₁₀ aryloxy, C₃-C₈ cycloalkyloxy, C₃-C₈ cycloalkyl C₁-C₆ alkoxy, C₂-C₂₂ alkenyloxy, C₃-C₈ cycloalkenyloxy, C₇-C₃₂ aralkyloxy, C₇-C₃₂ alkylaryloxy, C₉-C₃₂ aralkenyloxy, and C₉-C₃₂ alkenylaryloxy;

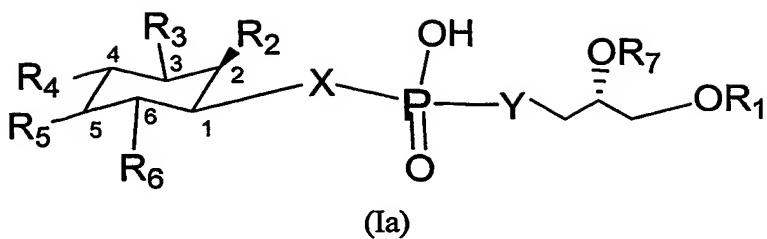
R₃-R₆ are independently selected from the group consisting of H, OH, isosteres of OH; and R₁ and R₇ are independently selected from the group consisting of C₁-C₂₅ alkyl, C₆-C₁₀ aryl, C₃-C₈ cycloalkyl, C₂-C₂₂ alkenyl, C₃-C₈ cycloalkenyl, C₇-C₃₂ aralkyl, C₇-C₃₂ alkylaryl, C₈-C₃₂ aralkenyl, and C₈-C₃₂ alkenylaryl;

with the provisos that (i) when X is O, Y is O or CH₂, and R₃ is H, at least one of R₂ and R₄-R₆ is not OH; (ii) when A is CH₂COOH or CH(COOH)₂, X and Y cannot be simultaneously O; and (iii) all of R₂-R₆ are not simultaneously H.

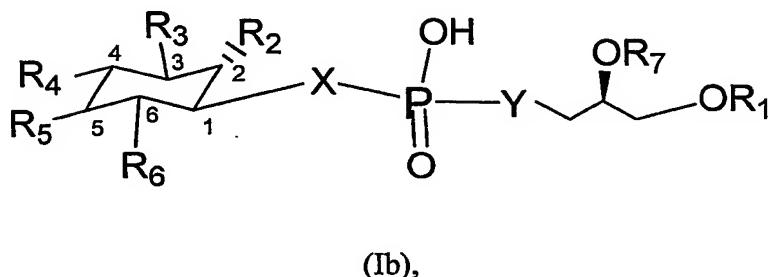
[0022] The alkyl and alkenyl portions of R₁-R₇ can be branched, or preferably linear. The aryl portion of R₁-R₇ can have one or more aromatic rings of 6-14 carbon atoms, for example, phenyl, naphthyl, or anthracyl rings. Isosteres of OH include F, Cl, SH, and the like.

[0023] In a preferred embodiment, A is P(O)OH. In a further preferred embodiment, where A is P(O)OH, both X and Y are O.

[0024] The stereochemistry of attachment to the respective carbon atoms of R₂ and OR₇ can be in any suitable form, e.g., each can be R, S, or a mixture of R and S forms. Thus, for example, the compound of the present invention can have the formula Ia:



or the formula Ib:



the formula Ia being more preferred.

[0025] In some embodiments, compounds of the present invention, particularly those of the formula Ia and Ib, have as R₁ a C₁-C₂₅ alkyl, preferably a C₁₀-C₂₅ alkyl, and more preferably a C₁₅-C₂₀ alkyl. Thus, for example, a particular R₁ is a C₁₈ alkyl, e.g., n-C₁₈H₃₇. In certain embodiments, the compounds of the present invention have as R₇ a C₁-C₂₅ alkyl, preferably a C₁-C₁₅ alkyl, more preferably a C₁-C₅ alkyl. Thus, for example, a particular R₇ is methyl. In particularly preferred embodiments, R₁ is a C₁₈ alkyl (e.g., n-C₁₈H₃₇) and R₇ is methyl.

[0026] In certain embodiments, compounds of the present invention, particularly those of the formula Ia and Ib, have as R₂ a C₁-C₂₅ alkyloxy, preferably a C₁-C₁₅ alkyloxy, more preferably a C₁-C₅ alkyloxy. A particular R₂ is methoxy. In certain other embodiments, R₂ is C₇-C₃₂ aralkyloxy, and in some other embodiments, R₂ is C₃-C₈ cycloalkyloxy, or C₃-C₈ cycloalkyl C₁-C₆ alkoxy, e.g., cyclohexylmethoxy.

[0027] In embodiments of the present invention, at least one of R₂, R₃, R₄, R₅, or R₆ is H, for example, R₂ and R₃ are H, R₃ and R₄ are H, or R₅ and R₆ are H.

[0028] In a preferred embodiment, A is P(O)OH, both X and Y are O, R₁ is C₁₈H₃₇, and R₇ is methyl. For example, in embodiments of the present invention, A is P(O)OH, both X and Y are O, R₁ is C₁₈H₃₇, and R₇ is methyl, and (i) R₂ is methoxy, R₃ is H, and R₄-R₆ are OH; (ii) R₂-R₃ are H and R₄-R₆ are OH; (iii) R₂-R₃ and R₅-R₆ are OH and R₄ is H; (iv) R₂ is i-butyl, R₃ is H, and R₄-R₆ are OH; (v) R₂ is cyclohexylmethoxy, R₃ is H, and R₄-R₆ are OH; (vi) R₂-R₃ and R₆ are OH and R₄-R₅ are H; (vii) R₂-R₄ and R₆ are OH and R₅ is H; or

(viii) R₂, R₄, and R₆ are OH and R₃ and R₅ are H. Some of the compounds of the present invention are shown in Figures 1A-1C.

[0029] The compounds of the present invention may be in the form of a pharmaceutically acceptable salt, for example, a salt of an alkali metal (e.g., sodium or potassium), alkaline earth metal (e.g., calcium), or ammonium of salt.

[0030] The present invention further provides a pharmaceutical composition comprising a compound described above and a pharmaceutically acceptable carrier. The pharmaceutically acceptable (e.g., pharmacologically acceptable) carriers include, for example, vehicles, adjuvants, excipients, or diluents, and are well-known to those who are skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active compounds and one which has little or no detrimental side effects or toxicity under the conditions of use.

[0031] The choice of carrier will be determined in part by the particular active compound, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the pharmaceutical composition of the present invention. The composition may be administered in any suitable formulation, for example, as a formulation for oral, aerosol, parenteral, subcutaneous, intravenous, intraarterial, intramuscular, interperitoneal, intrathecal, rectal, or vaginal administration.

[0032] Formulations suitable for oral administration can comprise (a) liquid solutions, such as an effective amount of the compound dissolved in diluents, such as water, saline, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient (compound), as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations can include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant, suspending agent, or emulsifying agent. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and

acacia, emulsions, gels, and the like containing, in addition to the active ingredient, such carriers as are known in the art.

[0033] The compounds of the present invention, alone or in combination with other suitable components, can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also can be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer. Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The compound can be administered in a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol, isopropanol, or hexadecyl alcohol, glycols, such as propylene glycol or polyethylene glycol, glycerol ketals, such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, such as poly(ethyleneglycol) 400, an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

[0034] Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters. Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl- β -aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

[0035] The parenteral formulations will typically contain from about 0.5 to about 25% by weight of the active ingredient in solution. Suitable preservatives and buffers can be used in

such formulations. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants. The quantity of surfactant in such formulations typically ranges from about 5 to about 15% by weight. Suitable surfactants include polyethylene or polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0036] The compounds of the present invention may be made into injectable formulations. The requirements for effective pharmaceutical carriers for injectable compositions are well known to those of ordinary skill in the art. See Pharmaceutics and Pharmacy Practice, J.B. Lippincott Co., Philadelphia, PA, Bunker and Chalmers, eds., pages 238-250 (1982), and ASHP Handbook on Injectable Drugs, Toissel, 4th ed., pages 622-630 (1986).

[0037] Additionally, the compounds of the present invention may be made into suppositories by mixing with a variety of bases, such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0038] The present invention further provides a method of preventing or treating a disease, or a condition that predisposes to a disease, that is characterized by or caused by the activation of the serine/threonine kinase Akt in an animal comprising administering to the animal a preventive or treatment effective amount of a compound described above. An example of a disease or condition is cancer. Particular examples of cancer include breast cancer, lung cancer, ovarian cancer, uterine cancer, brain cancer, sarcoma, melanoma, leukemia, lymphoma, colorectal cancer, prostate cancer, and liver cancer. Another disease or condition is rheumatologic disease, e.g., rheumatoid arthritis or osteoarthritis. A further example of the disease or condition is pulmonary disease, e.g., chronic obstructive pulmonary disease (COPD). The present invention further provides a method of increasing apoptosis of a cell, e.g., cancer cell, comprising contacting or treating the cell with a compound described above. The compounds of the present invention can be used as scientific tools to determine the presence of a disease or condition that are characterized by Akt activation.

[0039] They could be used alone or combined with other types of therapies to treat disease. Diseases characterized by Akt activation that could benefit from administration of the compounds include all forms of cancer, precancerous lesions, cardiovascular disease, rheumatologic disease, pulmonary disease, dermatologic disease, gynecological diseases, vascular disease, neurologic disease, and infectious disease, including bacterial, viral, retroviral, and parasitic diseases. Moreover, these compounds could be utilized to prevent above said diseases. Assays incorporating these compounds could provide predictive or prognostic value to patients with above said diseases or conditions.

[0040] Suitable doses and dosage regimens can be determined by conventional range-finding techniques known to those of ordinary skill in the art. Generally, treatment is initiated with smaller dosages, which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired. In proper doses and with suitable administration of certain compounds, the present invention provides for a wide range of responses. Typically the dosages range from about 0.001 to about 1000 mg/kg body weight of the animal being treated/day. Preferred dosages range from about 0.01 to about 10 mg/kg body weight/day, and further preferred dosages range from about 0.01 to about 1 mg/kg body weight/day.

[0041] The present invention further provides a method for inhibiting PH domain binding comprising exposing a material containing an PH domain to a compound described above. The present invention further provides a method for determining the presence of a PH domain in a material comprising:

- (a) exposing a sample of the material to a PH domain binding compound and obtaining a first binding result;
- (b) exposing another sample of the material to a compound of the present invention and obtaining a second binding result; and
- (c) comparing the first and second binding results to determine whether a PH domain is present in the material.

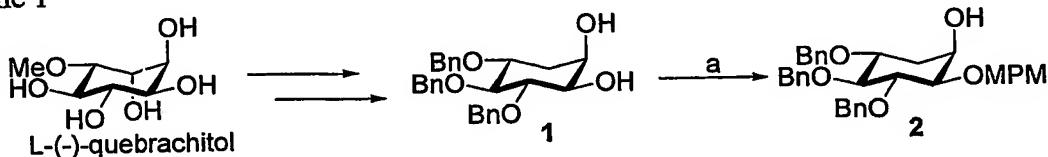
[0042] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0043] This Example illustrates a method of preparing precursors for 2-modified analogues.

[0044] This illustrates a method of preparing precursor for 3-deoxy 2-modified analogues. Compound **1** was prepared from L-(-)-quebrachitol according to a published method (*Tetrahedron*, 53, 14903-14914 (1997)). Selective *p*-methoxybenzylolation of the 1-OH in **1** via a 1,2-*O*-stannylene intermediate gave compound **2** (Scheme 1).

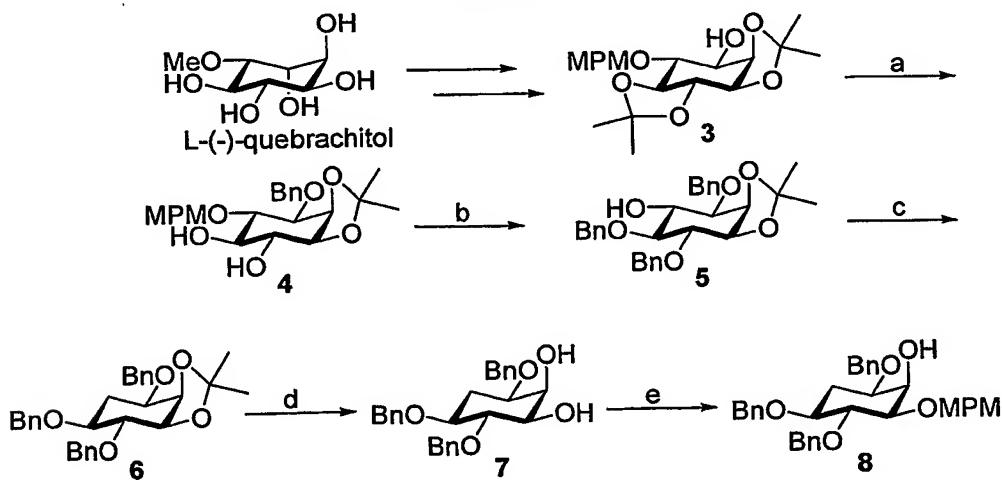
Scheme 1



Reagents and conditions: Bu_2SnO , toluene, reflux, then *p*-MeOC₆H₄CH₂Cl, CsF, DMF, rt, 92%.

[0045] This illustrates a method of preparing precursors for 4-deoxy 2-modified analogues. Compound **3** was prepared from L-quebrachitol (*Org. Lett.* 2, 115-117 (2000)). After benzyl protection of the 3-OH group, the trans-acetonide was selectively removed to give diol **4**. The two hydroxyl groups of **3** were protected by benzylation, and the MPM group at position 4 was removed by oxidation with ceric ammonium nitrate (CAN) to yield alcohol **5**. Barton-McCombie deoxygenation at C-4 gave compound **6**. Cleavage of the remaining acetonide in **6** gave diol **7**. Selective *p*-methoxybenzylolation of the 1-OH of **7** in the same method as **2** gave compound **8** (Scheme 2).

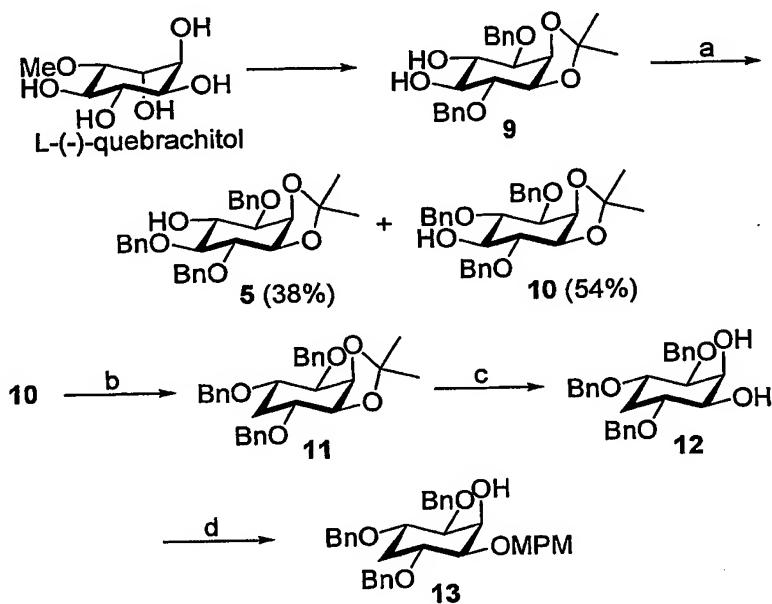
Scheme 2



Reagents and conditions: (a) (i) NaH, BnBr, DMF, 0 °C – rt; (ii) AcCl, CH₂Cl₂-MeOH 4:1 (v/v), rt, 86% for two steps; (b) (i) NaH, BnBr, 0 °C - rt, DMF; (ii) CAN, CH₃CN-H₂O 4:1 (v/v), 0 °C - rt, 84% for two steps; (c) (i) NaH, CS₂, 0 °C, DMF, then MeI; (ii) Bu₃SnH, AIBN, toluene, reflux, 95% for two steps; (d) AcCl, MeOH, rt, 98%; (e) Bu₂SnO, toluene, reflux, then *p*-MeOC₆H₄CH₂Cl, CsF, DMF, rt, 93%.

[0046] This illustrates a method of preparing a precursor for 5-deoxy-2-modified analogues. Compound 9 was synthesized according to a literature method (*Org. Lett.*, 2, 115-117 (2000)). Mono-benzylation of the diol moiety of 9 via a 4,5-O-stannylene intermediate gave two compounds 5 and 10 in a ratio of 1:1.5, which were separated by careful chromatography on silica gel. Barton-McCombie deoxygenation at C-5 of compound 10 gave compound 11. Cleavage of the trans-acetonide yielded diol 12. Selective p-methoxybenzylolation of the 1-OH of 12 in the same method as 2 gave compound 13 (Scheme 3).

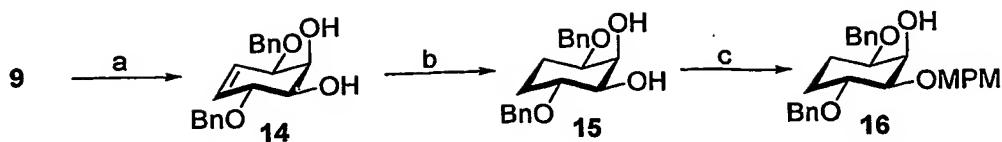
Scheme 3



Reagents and conditions: (a) Bu_2SnO , toluene, reflux, then BnBr , CsF , DMF, rt; (b) (i) NaH , CS_2 , 0 °C, DMF, then MeI ; (ii) Bu_3SnH , AIBN, toluene, reflux, 92% for two steps; (c) AcCl , MeOH , rt, 98%; (d) Bu_2SnO , toluene, reflux, then $p\text{-MeOC}_6\text{H}_4\text{CH}_2\text{Cl}$, CsF , DMF, rt, 92%.

[0047] This illustrates a method for preparing precursors for 4,5-dideoxy-2-modified analogues. Removal of the two hydroxyl groups in 9 by Barton-McCombie deoxygenation, followed by removal of the trans-acetonide gave olefinic diol 14. Selective hydrogenation of the C = C double bond catalyzed by 5% Pd-C in ethyl acetate gave diol 15. Selective *p*-methoxybenzylolation of the 1-OH of 15 in the same method as 2 give compound 16 (Scheme 4).

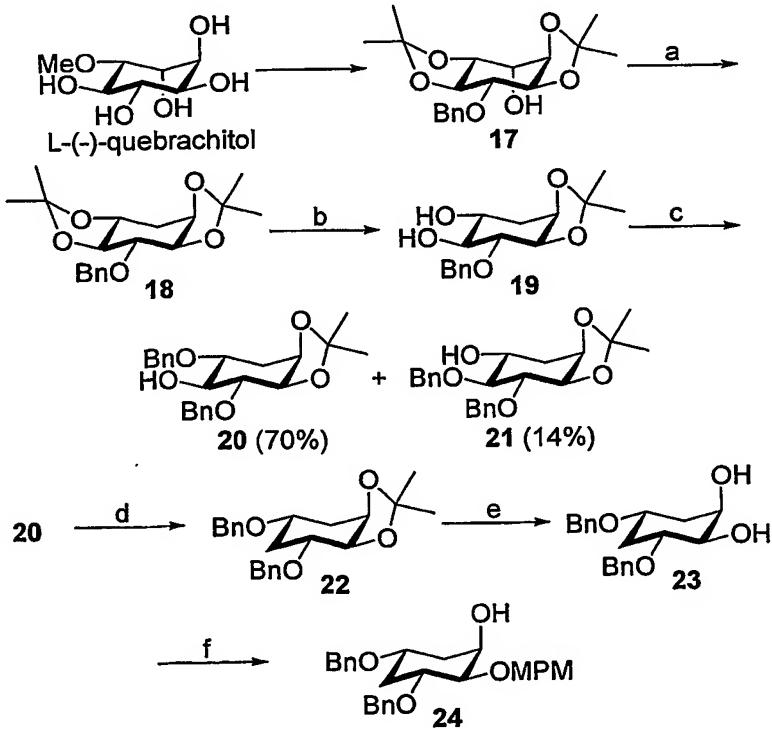
Scheme 4



Reagents and conditions: (a) (i) NaH, CS₂, 0 °C, DMF, then MeI; (ii) Bu₃SnH, AIBN, toluene, reflux; (iii) AcCl, MeOH, rt, 64% for three steps; (b) H₂, 5% Pd-C, ethyl acetate, rt, 1 atm, 95%; (c) Bu₂SnO, toluene, reflux, then *p*-MeOC₆H₄CH₂Cl, CsF, DMF, rt, 92%.

[0048] This illustrates a method for preparing precursors for 3,5-dideoxy-2-modified analogues. Compound 17 was synthesized according to a published procedure (*Org. Lett.* 2, 115-117 (2000)). Barton-McCombie deoxygenation at C-3 furnished compound 18. Selective cleavage of the trans-acetonide gave diol 19. Mono-benzylation of the diol moiety gave two compounds 20 and 21 in a ratio of 5:1. The structure of compound 21 was confirmed by transformation into a known intermediate in the synthesis of a 3,4-dideoxy PI analogue (*Tetrahedron Lett.*, 41, 7415-7418 (2000)). Barton-McCombie deoxygenation at C-5 in intermediate 20 gave compound 22. Cleavage of the second acetonide yielded diol 23. Selective *p*-methoxybenzylolation of the 1-OH of 23 in the same method as 2 give compound 24 (Scheme 5).

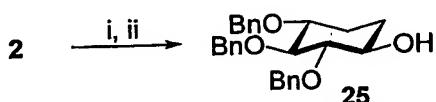
Scheme 5



Reagents and conditions: (a) (i) NaH, CS₂, 0 °C, DMF, then MeI; (ii) Bu₃SnH, AIBN, toluene, reflux, 94% for two steps; (b) AcCl, CH₂Cl₂-MeOH 4:1 (v/v), rt, 86%; (c) Bu₂SnO, toluene, reflux, then BnBr, CsF, DMF, rt; (d) (i) NaH, CS₂, 0 °C, DMF, then MeI; (ii) Bu₃SnH, AIBN, toluene, reflux, 93% for two steps; (e) AcCl, MeOH, rt, 96%; (f) Bu₂SnO, toluene, reflux, then *p*-MeOC₆H₄CH₂Cl, CsF, DMF, rt, 93%.

[0049] Barton-McCombie deoxygenation at C-2 in compound **2** followed by removal of the MPM group on 1-OH gave compound **25** (Scheme 6).

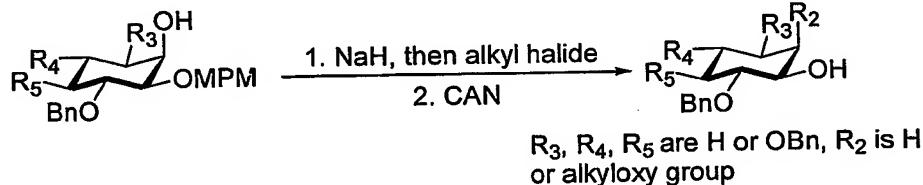
Scheme 6



Reagents and conditions: (i) NaH, CS₂, then MeI, 0 °C, DMF; (ii) Bu₃SnH, AIBN, toluene, reflux; (iii) CAN, CH₃CN-H₂O 4:1 (v/v), 0 °C – rt, 81% over two steps.

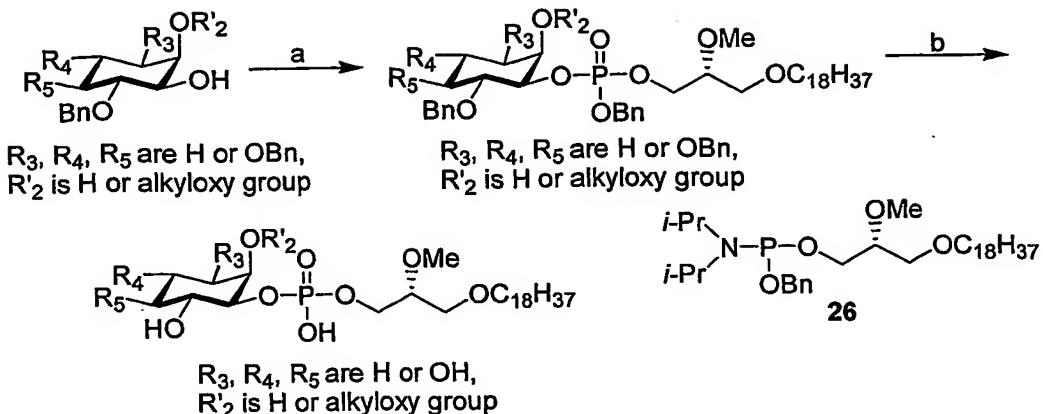
[0050] This illustrates a method of modifying position 2. The above precursors were alkylated with suitable alkyl halide to give a series of 2-modified compounds. The MPM group in these compounds was cleaved by oxidation with CAN to give a series of precursors for phosphorylation (Scheme 7).

Scheme 7



[0051] The above precursors were phosphorylated by reaction with the ether lipid phosphoramide **26** catalyzed by 1*H*-tetrazole and subsequent oxidation of the phosphite intermediates with *m*-CPBA to give a series of phosphates. Finally, these phosphates were completely deprotected by catalytic hydrogenation to give the desired analogues (Scheme 8).

Scheme 8



Reagents and conditions: (a) (i) 26, 1*H*-tetrazole, rt, CH₂Cl₂; (ii) *m*-CPBA, 0 °C – rt, CH₂Cl₂; (b) H₂, 20% Pd(OH)₂-C, *t*-BuOH, rt, 1 atm.

EXAMPLE 2

[0052] This Example illustrates some of the properties of the compounds of the present invention. Figure 1D shows results of an immunoblotting experiment performed with antibodies against phosphorylated Akt at S473 and at native Akt after individual administration of the compounds to cancer cell lines. Phospho-specific antibodies only recognize kinases in an active state. Two cell lines were used that have high levels of constitutively active Akt, H1703 and H157. H1703 has wild type PTEN and H157 has mutant PTEN. Compounds SH5, 6, 13, 16, 23, 24, 25 decreased Akt phosphorylation without affecting native Akt levels.

[0053] All but SH16 also decreased Akt phosphorylation in the H157 cells. The lack of decreased phosphorylation observed with SH7 is important because SH7 is composed of only the ether lipid portion of these analogues and thus serves as a negative control. This data indicate that inhibition of Akt phosphorylation by some of the SH compounds is cell line specific, and that inhibition by SH5, 6, 13, 23-25 is not dependent on PTEN. Figure 1E shows that SH5, 6, 23-25 inhibit Akt kinase activity, as well as Akt phosphorylation.

[0054] Figure 1F shows results on some of the compounds. SH5, 6, 23, 24, and 25 completely inhibited Akt phosphorylation in all three cell lines and were chosen for further analysis.

[0055] Figure 2A is a set of representative immunoblotting experiments with increasing doses of SH5, 6, and 24. For each set of immunoblotting experiments, densitometry was performed to quantify the decreased intensity of the bands observed with the S473 antibodies. Quantitative inhibition of S473 phosphorylation by different doses of these

compounds is shown in Figure 2B. The IC₅₀ values for these compounds (including SH23 and 25 (data not shown)) were similar, between 2-4 μ M.

[0056] The SH compounds were tested against Akt and other kinases that are either upstream of Akt (PDK-1), downstream of Akt (c-Raf, 4EBP-1, p70S6K, FKHR, GSK-3, and/or AFX), or downstream of Ras (ERK, p38). Immunoblotting was carried out with phospho-specific antibodies to assess activation state of the kinases, and with native antibodies to assess changes in protein levels. Similar results were obtained with the H1703 (Figure 3A) or H157 cells (Figure 3B). SH5, 6, and 10 inhibited Akt phosphorylation without affecting native Akt levels. DPIEL and SH7 did not decrease Akt phosphorylation. Upstream of Akt, phosphorylation of PDK-1 was not affected by any SH compound. Of the downstream substrates, c-Raf phosphorylation and 4EBP-1 phosphorylation were decreased most. Decreased c-Raf phosphorylation correlated with increased ERK and p38 phosphorylation, which is consistent with the inhibitory effect of S259 phosphorylation by Akt on c-Raf activity. Of note, p38 phosphorylation was only decreased by DPIEL, indicating that the DPIEL used in these experiments was not inert.

[0057] SH 23-25 had similar effects in both H1703 (Figure 3C) and MB468 (Figure 3D) cells (H157 data not shown). In H1703 cells, phosphorylation of Akt, c-Raf, and 4-EBP-1 was decreased by SH23-25, but PDK-1 phosphorylation was unaffected. MB468 cells decreased Akt phosphorylation with administration of SH23-25 without decreasing PDK-1 phosphorylation. Some effects of SH23-25 were unique to the MB468 cells. Because MB468 cells had little endogenous phosphorylated c-Raf, we could not evaluate inhibition of c-Raf. Phosphorylation of 4EBP-1 was not affected by SH23-25, but phosphorylation of GSK-3 was decreased.

[0058] Cell lines containing high or low endogenous levels of Akt activity were treated with SH compounds and the cell apoptosis was measured. As shown in Figure 4, three cell lines with high levels of Akt activity, H1703, H157, and H1155 cells, increased apoptosis by 10-20 fold with administration of SH5 or 6. Increased apoptosis was also observed with administration of SH5 or 6 in MB468 cells (data not shown). SH7 had no effect on apoptosis, similar to DPIEL (data not shown). In contrast to cells with high levels of Akt, cells with low endogenous Akt levels (H1355 and A549 cells) did not undergo apoptosis in response to SH5, 6, or 7.

[0059] SH5, 6, 23, 24, and 25 are very active in decreasing Akt phosphorylation in a panel of cancer cell lines. These compounds decrease Akt phosphorylation as well as Akt kinase activity, with IC₅₀s in the low micromolar range. These compounds appear to be specific for Akt, as the phosphorylation of the upstream kinase, PDK-1, is unaffected by any of the SH compounds, as is phosphorylation of ERK and p38, which are downstream of

Ras. Cell-line specific, selective inhibition of phosphorylation of substrates downstream of Akt, such as c-Raf, 4EBP-1, GSK-3, and/or FKHR/AFX was observed. These compounds selectively increased apoptosis in cancer cell lines that depend on Akt activity for survival. Phosphorylation of tuberin, 4EBP-1 and P70S6K (substrates that control protein translation) was inhibited by SH23, 24, and 25; and SH5-6 inhibited phosphorylation of tuberin and 4EBP-1. Phosphorylation of forkhead family members AFX and FKHR that control transcription was inhibited by SH23 or 25. Phosphorylation of GSK-3 and c-Raf was attenuated by SH23-25.

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[0060] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0061] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely

intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0062] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.